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The Antibacterial Activity of Polyherbal Extract, Its Pharamaceutical Formulation Development and Evaluation Studies

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ABSTARCT

An antibiotic is a substance produced by one microorganism that selectively inhibits the growth of another. Synthetic antibiotics, usually chemically related to natural antibiotics, have since been produced that accomplish comparable tasks. Unlike pharmaceutical medicines, herbal medicines are complex biological creatures that have evolved slowly over time and contain hundreds, if not thousands, of active compounds that all work together synergistically. Pharmaceutical antibiotics are, in fact, simple substances, not complex, and because of this, bacteria can more easily figure out how to counteract their effects, but herbs like garlic are very complex".Polyherbal formulation are known to express high effectiveness in vast no of diseases. They are usually found to have wide theraupetic range. Often, it results in few side effects as compared to allopathic drugs. The present research work was carried out to prove the antibacterial activities of polyherbal extract include Phyllanthus emblica, Aloevera, Eclipta alba, Tinospora cardifolia, Sphaeranthus indicus which can used as an alternative for synthetic medicine to cure bacterial infections. The crude leaves were collected from local forest which was then subjected for extraction was done by using maceration process. The effect of antibacterial activity of polyherbal extract is studied by using both gram positive and gram negative strains like E.coli, S.aureus ,K.pnuemonia and S. typhii They were used as test organisms. From the agar diffusion results was confirmed, that the extract may have antibacterial activities. Moreover, these plants extract should be investigated in vitro to better understand their safety, efficacy and properties. This extract used to prepare syrup, physical properties, viscosity- 156 centipoises, pH- 5.15, and specific gravity - 1.321.

Keywords: Antibacterial activity, Pharmaceutical syrup, Herbal antibiotics.

INTRODUCTION

A drug used to treat infections caused by bacteria and other microorganisms. Originally, an antibiotic was a substance produced by one microorganism that selectively inhibits the growth of another. Synthetic antibiotics, usually chemically related to natural antibiotics, have since

been produced that accomplish comparable tasks [1]. Herbal antibiotics have long been used by herbal healers to ward off colds and flu, clear infections and speed wound healing. Now, they may be moving back into the mainstream as an alternative for bacteria that have become resistant to synthetic antibiotics. This post is based on the book "Herbal Antibiotics" by Stephen Harrod Buhner, and related materials. We'll start with some background information and then discuss antibiotic herbs and their use.

Most of us think of antibiotics as liquid or pills you pick up at the pharmacy, but these compounds were originally developed from naturally occurring sources. Plants have antibiotic substances serving a beneficial roll around their root systems. Bacteriophages are viruses that infect bacteria. (Everything has something that wants to eat it.) Many common foods and herbs (and some not so common ones) act as antibiotics, such as honey, garlic, onions, licorice root, ginger, sage and many others [5].

Herbal antibiotics different from pharmaceutical antibiotics

Many pharmaceutical antibiotics are isolated chemical constituents. They are one compound/one chemical – penicillin is penicillin, tetracycline is tetracycline and so on. This makes them easier for bacteria to adapt to and counteract. In contrast, herbs are much more complicated. Garlic has over 33 sulfur compounds, 17 amino acids and a dozen other compounds. Yarrow has over 120 identified compounds. (It makes me look at my herbs with a new appreciation.) In plants, the whole appears to be more than the sum of its parts. The different compounds work together, often to produce better than expected results.

Top 15 Antibiotic Herbs

Stephen lists the following as his top 15 antibiotic herbs. I might also add cinnamon (perhaps it's been added in his second edition, which I haven't had the pleasure of reading yet).

Acacia,	Aloe,	Cryptolepsis,	Echinacea,	Eucalyptus,
❖ Garlic, l,	Ginger,	Goldensea	Grapefruit Seed Extract	Honey
❖ Juniper,	Licorice,	Sage,	Usnea,	❖ Wormwood

For the treatment of diseases inhibitory chemicals employed to kill micro-organisms or prevent their growth, are called antimicrobial agents. These are classified according to their application and spectrum of activity, as germicides that kill micro-organisms, whereas micro-biostatic agents inhibit the growth of pathogens and enable the leucocytes and other defense mechanism of the host to cope up with static invaders. The germicides may exhibit selective toxicity depending on their spectrum of activity. They may act as viricides (killing viruses), bacteriocides (killing bacteria), algicides (killing algae) or fungicides (killing fungi).

The beginning of modern chemotherapy has largely been due to the efforts of Dr. Paul Ehrlich (1910), who used salvarsan, as arsenic derivative effective against syphilis. Paul Ehrlich used the term chemotherapy for curing the infectious disease without injury to the host's tissue, known as chemotherapeutic agents such as antibacterial, antiprotosoal, antiviral, antineoplastic, antitubercular and antifungal agents. Later on, Domagk (1953) prepared an important chemotherapeutic agent sulfanilamide.

Consumption of antibiotics is linked to bacterial resistance. In hospitals, most common resistant bacteria include methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci and gram-negative rods, including the Enterobacteriaceae and Pseudomonas aeruginosa [4].

Many medicinal plants are considered to be potential antimicrobial crude drugs as well as a source for novel compounds with anti-microbial activity, with possibly new modes of action. This expectation that some naturally occurring plant compounds can kill antibiotic-resistant strains of bacteria such as Bacillus cereus, Escherichia coli, Micrococcus luteus and S. aureus has been confirmed, for example, by Friedman et al. [2006].

In the past few decades, the search for new anti-infection agents has occupied many research groups in the field of ethnopharmacology. A Pubmed search for the antimmicrobial activity of medicinal plants producesd a 115 articles from the period between 1966 and 1994. However, in the following decade between 1995 and 2004, this number more than doubled, to 307. In these studies

one finds a wide range of criteria related to the discovery of antimicrobial compounds in plants. Many focus on determining the antimicrobial activity of plant extracts found in folk medicine, essential oils or isolated compounds such as alkaloids, flavonoids, sesquiterpene lactones, diterpenes, triterpenes or naphthoquinones. After detection of antimicrobial activity in the plant extract, some of these compounds were isolated or obtained by bioassay-guided isolation. Second block of studies focuses on the random screening of natural flora of a specific region or country and the third relevant group of papers is made up of in-depth studies of the activity of a plant or plant compound against a specific pathological microorganism [6].

Importance of herbal antibiotics

Microbes are the leading producers of useful natural products. Natural products from microbes and plants make excellent drugs. Significant portions of the microbial genomes are devoted to production of these useful secondary metabolites[3]. A single microbe can make a number of secondary metabolites, as high as 50 compounds. The most useful products include antibiotics, anticancer agents, immunosuppressants, but products for many other applications, e.g., antiviral, anthelmintic, enzyme inhibitors, nutraceuticals, polymers, surfactants, bioherbicides, and vaccines have been commercialized [7].

- In general, medicinal plants more effective against gram-positive than gram-negative bacteria.
- Main antibacterial constituents in medicinal plants are phenols. Plants appear to synthesize phenolic compounds in response pathogen and insect attack, UV radiation and wounding [8].
- Phenols from spices active against *Staph. aureus*, *Bacillus cereus*, *E. coli* & Salmonella. Flavonoids are largest group of phenols.

Use of herbal medicine reserves synthetic antibiotics for severe cases

Many common ailments such as sinus problems, sore throats, simple urinary tract infections and superficial wounds do not necessitate antibiotics in most cases.

These can be effectively treated with appropriate diet and lifestyle changes and expert botanical medicine care by trained 1herbalists.

AIM & OBJECTIVES

- To collect and extract the contents from leaves and plant parts using alcohol and hydroalcoholic solvents by maceration process.
- To study the antibacterial effect of plant extracts which include aloe vera, amla (phyllanthus emblica), bhringaraja (Eclipta alba), bodasaramu (spheranthus indicus), tippateega (tinospora cardifolia) by agar well diffusion method and determining zone of inhibition which is compared with the streptomycin as standard.

METHODOLOGY

Plant collection and Preparation of the plant extract

Fresh (Aloevera, Amla, Bringraj, Thippatega, Bodatharam) leaves and plant parts were collected randomly from the various areas of Hyderabad, India. The leaves and plant parts were separated and washed twice with double distilled water. The plant parts were shade dried for 3 to 4 weeks. The leaves and plant parts were then subjected to extraction.

Plant Extracts Preparation

Leaves, plant parts of (Aloevera, Amla, Bringraj, Thippatega, Bodatharam) were dried at room temperature for 48 hrs. Clean and dry Maceration Extraction process was taken. Then 25 gm of ground material and 70 ml of 95% dehydrated ethanol and 30 of distilled water was filled in the apparatus and it was allowed to run till the completion of 6 cycles. After that the extract was collected and filtered using of standard filter paper for 4 hours. The filtrate was dried using of rotatory vacuum evaporator and the volume of the crude extract was reduced to 90% of its volume. All the extracts were kept in refrigerator prior to using. This extract was considered as the pure (100mg/ml) concentration of the extract. The extract which contains required chemical constituents was subjected evaporation process using Rota evaporator for the complete evaporation of solvent material.

After the confirmation of required chemical constituents, the extracted powder material was subjected to specific tests for compounds using standard procedures. The extracted powder was

tested for its antibacterial properties using agar diffusion method.

DEVELOPMENT OF POLYHERBAL SYRUP

Preparation of Extracts

The collected plant materials (500g) were dried under shade, size reduced into coarse powder and macerated separately with 500ml of ethanol. After 7 days of maceration, both the extract was filtered out and concentrated under vacuum using rotary vacuum evaporator. The residue obtained was kept in a dessicator for the present study.

Preparation of Simple Syrup

Weigh accurately 66.7g of sucrose and transferred into clean and dry beaker and then add 100ml of distilled water to it, heated until sucrose completely dissolved with occasional stirring. Sufficient boiling water was added to produce 100 ml.

Preparation of Polyherbal Syrup

0.2 gram of each extracts of extract were dissolved in simple syrup IP and the volume was made up to 100 ml by adding benzoic acid(0.1%) as preservative IP and ascorbic acid(0.1%) as an antioxidant IP.

Evaluation of Formulated Polyherbal Syrup

- The polyherbal syrup was evaluated for various parameters such as physical appearance (Colour, Odour, and Taste), pH, weight/ml and viscosity.
- Evaluation of the organoleptic parameters of the polyherbal syrup revealed that the syrup was dark brown colour and had a pleasant odour and sweet taste

Method used in present research work

An UV-VIS spectrophotometric method based on the measurement of absorbance at 249 nm in phosphate buffer as stock solution was used in the present research work for the estimation of extract. For the estimation of extract in different aqueous fluids the stock solution was subsequently diluted to get a series of dilutions 2, 4, 6, 8 and 10 µg/mL of solution and the absorbance was measured at 249 nm (UVVIS spectrophotometer, SL-150, Elico) against the same dilution as blank.

ANALYTICAL DATA

Preparation of 7.4 ph phosphate buffer

a. 0.2 M Potassium Dihydrogn pthalate was prepared by disolving 27.218gms of Potassium Dihydrogn pthalate in 1000ml distilled water
b. 0.2 M sodium hydroxide was prepared by dissolving 8gms of sodium hydroxide in 1000ml distilled water

c. Preparation of 7.4 ph phosphate buffer

Measure 39.1ml of 0.2 m sodium hydroxide and 50 ml of 0.2M Potassium Dihydrogn pthalate taken into a 1000ml volumetric flask and make up the volume using distilled water.
Standard Graph in 7.4 ph phosphate buffer was plotted.

Standard graph of Amla

The standard wavelength of the all 3 stock solution is 255nm and prepare the serial dilutions like 0.5,1.0,1.5,2.0,2.5,3.0 and plot a graph using concentrations veres absorbances.
Standard graphs of Aloe vera, Tippatega, Bringraj and Bodatharam were plotted**.**

Preparation of the stock solutions

The standard wavelength of all 2 stock solutions is 230 and prepare serial dilutions like 0.3,0.6,0.9,1.2,1.5,1.8 and plot a graph using concentration veres absorbances.

Evaluation parameters for syrup formulations

Syrup formulations were evaluated for appearance, viscosity, pH and drug content uniformity.

Appearance

Appearance is one of the most important parameter of syrup formulations. All the formulations were evaluated for clarity by visual appearance (colour).

pH

The developed formulations were evaluated for pH by using Elico LI 120 pH meter and estimations carried out in triplicate.

Drug content uniformity

Drug content was estimated in the syrup formulation by weighing approximately 250mg of

the fill formulation into a 5ml volumetric flask. Few ml of ethanol was added and dissolved the extracts and the volume was made up to 5mlwith remaining ethanol. Samples were suitably diluted with 0.1N HCL and the samples were analyzed for extracts content by measuring absorbance at 249nm. The estimations were carried out in triplicate.

Rheological studies

Viscosity of all formulations was measured using a Brookfield DV-II + PRO viscometer. The formulations were taken in cup of Brookfield DV-II + PRO viscometer rotated with CP52 spindle. The angular velocity was fixed at 10- 100 rpm. The viscosity measurements were made in triplicate using fresh samples each time at room temperature.

Fig: ***BROOKFEILD VISCOMETER***

PROCEDURE FOR DETERMINING ANTIBACTERIAL ACTIVITY

Preparation of Nutrient agar

1. Beef extraction : 10 g
2. Sodium chloride : 5 g
3. Peptone : 10 g
4. Agar : 4.5g
5. Distilled water : 1 Lit.

The above constituents were weighed and dissolved in water. The mixture was warmed on water bath till agar dissolved. This was then sterilized in an autoclave at 15 lbs pressure and 121 oC for fifteen minutes. The sterilized medium (20 ml) was poured in sterilized Petri dishes under aseptic condition, allowing them to solidify on a plane table.

Experimental procedure

1. The plates were inoculated by dipping a sterile swab into inoculums. Excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube, above the level of the liquid.
2. The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60 oC after each application. Finally the swab was passed round

the edge of the agar surface. The inoculation was dried for a few minutes, at room temperature, with the lid closed.

3. Ditch the bore in plate. Add compounds (extractions) solution in bore.
4. The plates were placed in an incubator at 370C within 30 minutes of preparation for bacteria
5. After 48 hrs incubation for bacteria, the diameter of zone (including the diameter disc) was measured and recorded in mm. The measurements were taken with a ruler, from the bottom of the plate, without opening the lid.

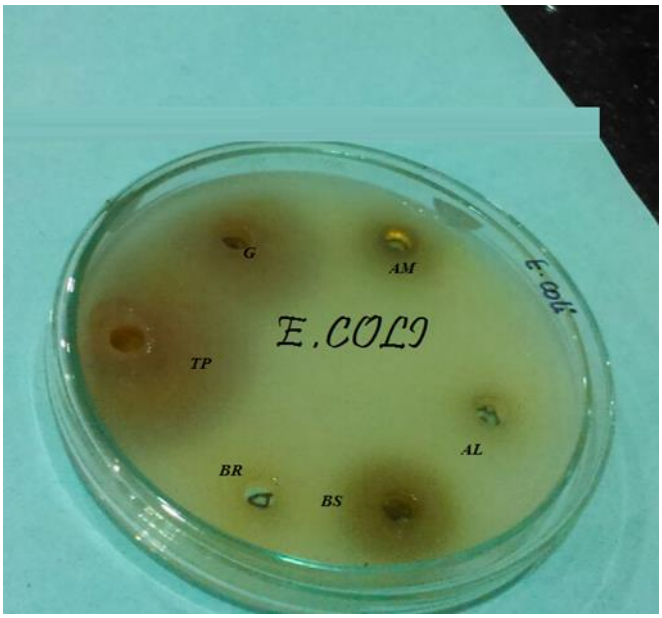
Study Of Antimicrobial Property

Various methods have been used from time to time by several workers to evaluate the antimicrobial activity. The evaluation can be done by the following methods:

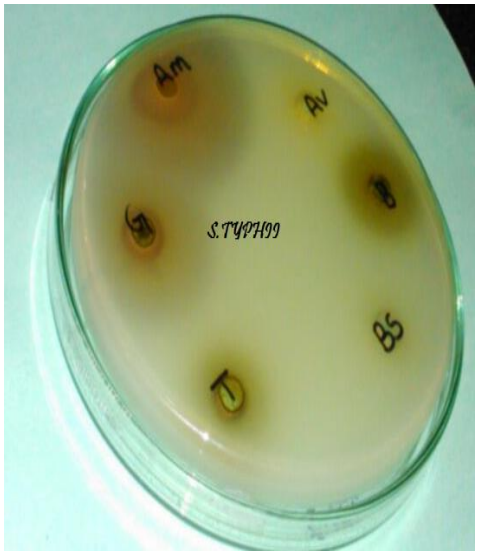
- Turbidometric method
- Agar streak dilution method
- Serial dilution method
- Agar diffusion method.

Following Techniques are used as agar diffusion method

Agar Cup method
Agar Ditch method
Paper Disc method

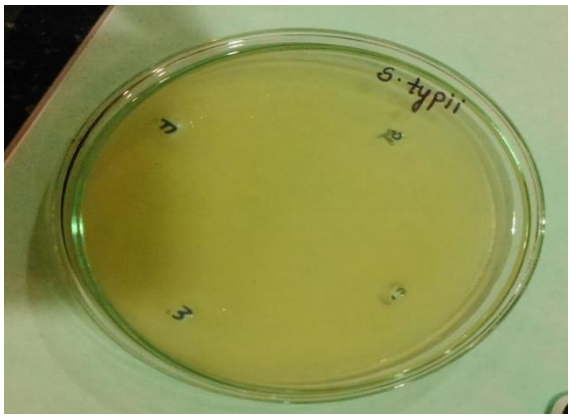


Escherichia coli-gram negative

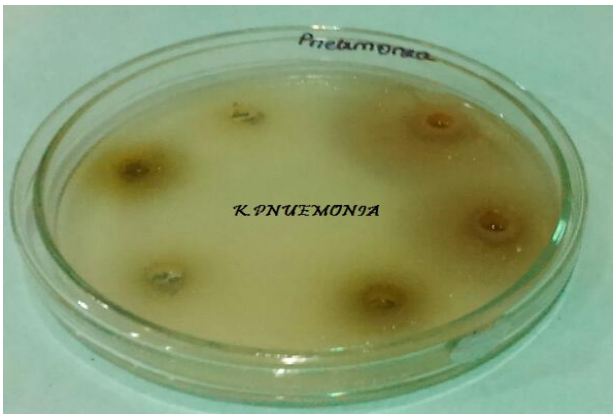


Salmonella typhi-gram negative

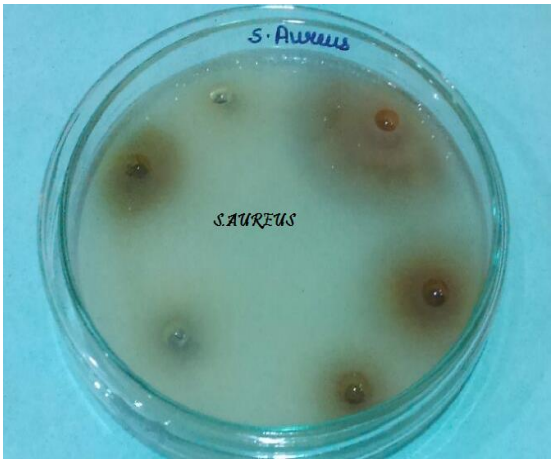
Escherichiacoli-gram negativ



Salmonella typi-gram negative



klebsiella pneumonia-gram negative



Staphylococcus aureus-gram positive

Table: Zone of inhibitions

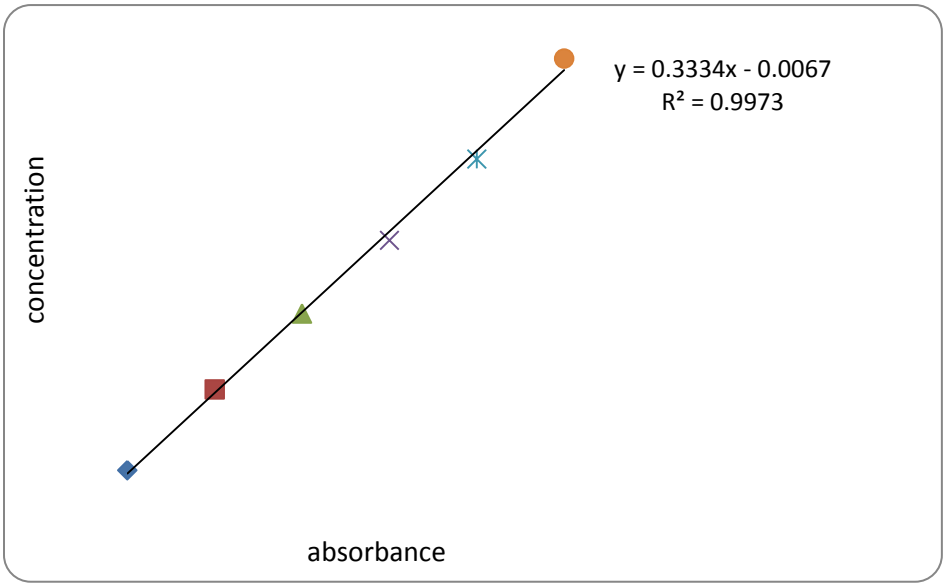
Org	Name of the extract									
	Amla	Aloevera	Bringraj	Thippa thega	Bodath aram	Strepto mycin	Ethanol	Distilled water	DW+ethanol	group
K. Pneumonia	30 mm	17 mm	19 mm	15 mm	16 mm	30 mm	2 mm	-	33 mm	20 mm
E.Coli	31 mm	12 mm	19 mm	16 mm	12 mm	25 mm	19 mm	19 mm	16 mm	22 mm
S. Typpi	13 mm	12 mm	10 mm	21 mm	10 mm	30 mm	25 mm	-	23 mm	19 mm
S. Auris	40 mm	19 mm	19 mm	11 mm	20 mm	25 mm	-	-	2 mm	20mm

Analytical Method

Graphs of Plant extracts was taken in Simulated Gastric fluid (pH 1.2) and in p H 7.4 phosphate buffer at 228 nm and 232 nm respectively.

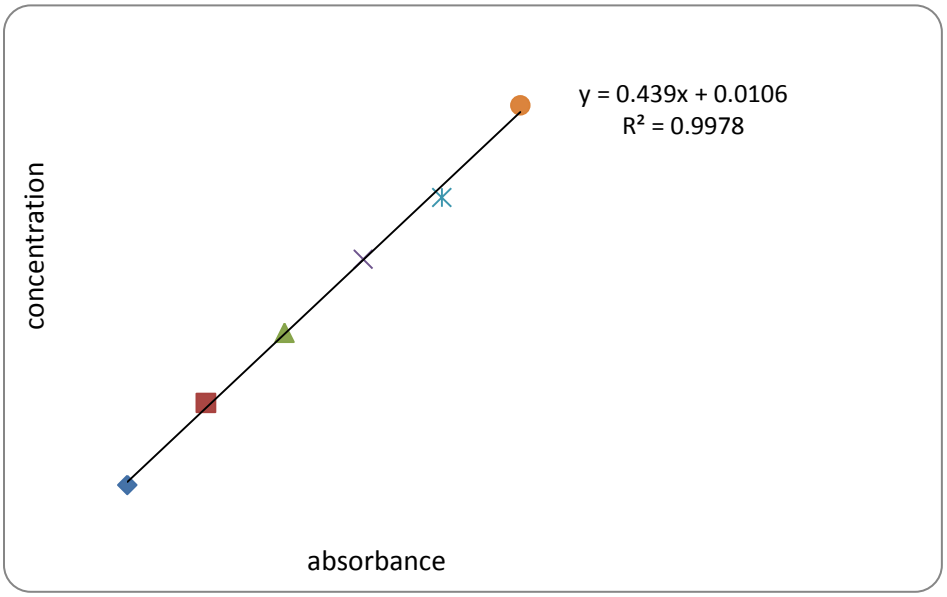
Standard graph of Bhringraj(320nm)

Concentration	Absorbance
0.5	0.167
1	0.324
1.5	0.475
2	0.643
2.5	0.851



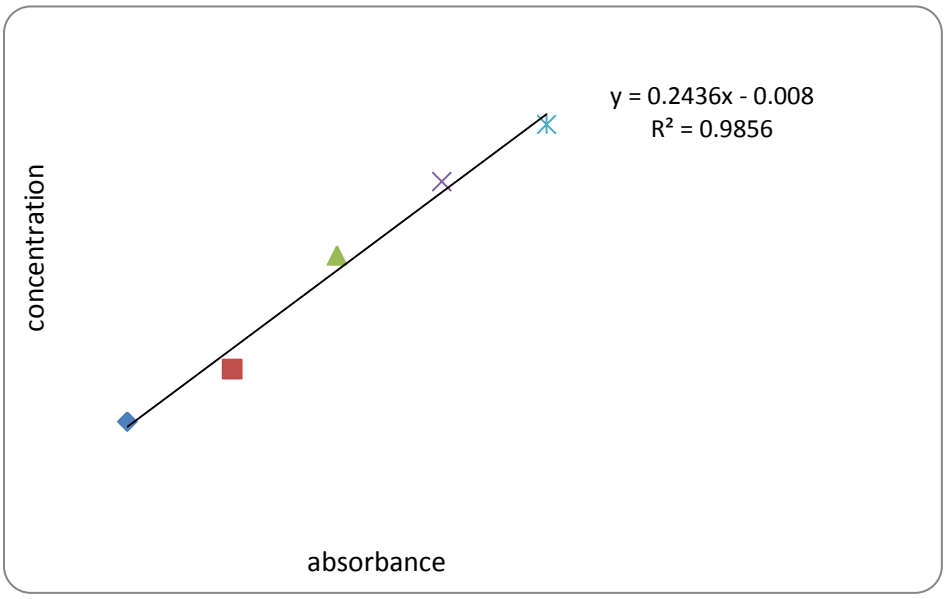
Standard graph of Aloevera(255nm)

Concentration	Absorbance
0.6	0.292
1.2	0.542
1.8	0.804
2.4	1.024
3.0	1.352



Standard graph of Amla(255nm)

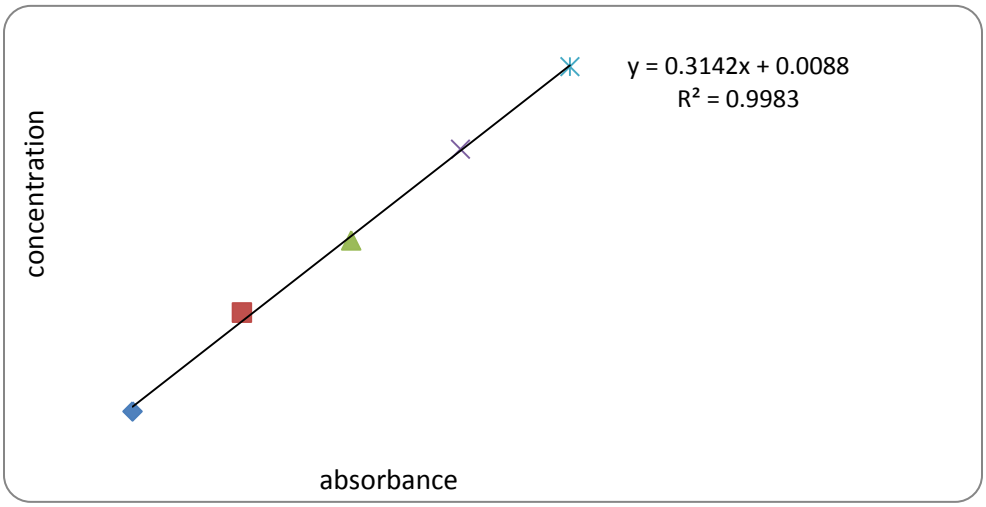
Concentration	Absorbance
0.5	0.082
1	0.259
1.5	0.374
2	0.463
2.5	0.572



Standard graph of Thippathega(275nm)

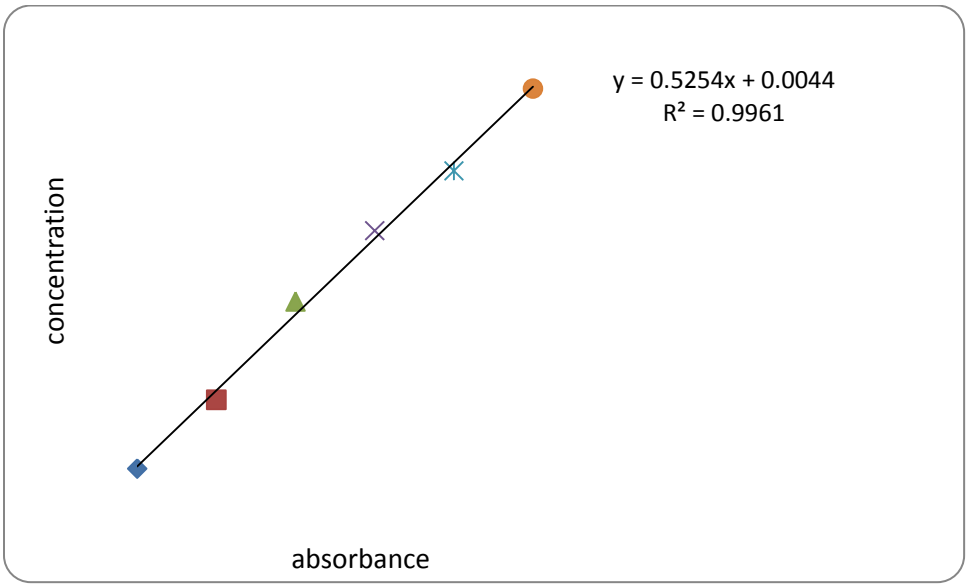
Concentration	Absorbance
0.5	0.182
1	0.315

1.5	0.483
2	0.635
2.5	0.779



Standard graph of Bodasaram(230nm)

Concentration	Absorbance
0.3	0.016
0.6	0.347
0.9	0.494
1.2	0.618
1.5	0.789
1.8	0.900



Evaluation of syrup [9, 10]

The prepared oral liquid herbal formulation showed good elegance and palatability. The formulated polyherbal syrup was evaluated for measurement of pH, specific gravity and stability. The final formulation found to have pH 5.15, viscosity 156 centipoise and specific gravity 1.321 g/ml. The results of stability study of formulated polyherbal syrup indicate the homogeneity of syrup without turbidity at storage temperature. The result of the antibacterial activity showed the single extract of the plant has significant anbacterial activity. Thus it can be concluded that these formulated polyherbal syrup could be suitable dosage form from leaves of aloevera, amla, bringraj , thippatega, bodatharam

PHYSICOCHEMICAL PARAMETERS	RESULTS
Colour	Brown
Taste	sweet
Odour	Pleasant
pH	5.15
Viscosity	156
Specific gravity	1.321

*Result of physicochemical parameters of developed poly herbal syrup

Sample..	Time duration..	Temperature(^{0}C)	Colour..	Odour	Taste	Specific gravity	Viscoisty..	pH	Turbidity.
1A	24hr	Room temp	NC	NC	NC	1.321	156	5.15.	No turbidity
1B	24hr	Room temp	NC	NC	NC	1.321	156	5.15	No turbidity
2A	48hr	Room temp	NC	NC	NC	1.321	156	5.15	No turbidity
2B	48hr	Room temp	NC	NC	NC	1.321	156	5.15	No turbidity
3A	72hr	Room temp	NC	NC	NC	1.321	156	5.15	No turbidity
3B	72hr	Room temp	NC	NC	NC	1.321	156	5.15	No turbidity
4A	96hr	Room temp	NC	NC	NC	1.321	156	5.15	No turbidity
4B	96hr	Room temp	NC	NC	NC	1.321	156	5.15	No turbidity
5A	120hr	Room temp	NC	NC	NC	1.321	156	5.14	No turbidity
5B	120hr	Room temp	NC	NC	NC	1.321	156	5.14	No turbidity

RESULTS & DISCUSSION

Plant collection and Preparation of the plant extract

The dried leaves of subjected to extraction process using ethanol as solvent**.** The extract was analyzed for the presence of ascorbic acid which is responsible for antibacterial activity.

Disc diffusion method

The antibacterial activity of test compound was estimated by disc diffusion method. In this streptomycin was taken as standard antimicrobial compound. E.coli (Gram negative bacteria). Staphylococcus aureus, klebsillus pnuemonia and salmonella typhii (were taken as test organisms).

CONCLUSION

- The results of this work suggest that the compound extracted from leaves have a broad spectrum of antibacterial activity and this effect is increased by increasing the quantity of this compound, which can be used as an alternative for antibiotics. The crude leaves were collected from local forest which are then subjected for drying, extraction using maceration process. To prove the antibacterial activity of the extracts we have done the zone of inhibition E.coli, S.aureus ,S.pnemonia and S. thyppi were used as test organisms by employing the agar diffusion method. From the agar diffusion results it was confirmed that the extract may have both antibacterial and antifungal activities.Then we

performed lambda max by using 7.4ph phosphate buffer solution because to prove that our drug has absorbance and prepared standard graphes by ploting a graph concentration veres absorbances. Therefore, pharmacological test is necessary to isolate and characterize their active compounds. Moreover, these plants extract should be investigated in vivo to better understand their safety, efficacy and properties.

- The extract used to prepare syrup, and the syrup was evaluated for the:
- Physical properties(colour,taste,odour,clarity)
- viscosity- 156 centipoises, (by using Brookfield viscometer)
- pH- 5.15(was done 1^{st},2^{nd},3^{rd}.....15^{th}day),
- specific gravity- 1.321
- The % drug release of syrup is 87.86% in 300min.

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